Donated Chemical Probe

CCR1 Antagonist Probe BAY-3153

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Presenters:
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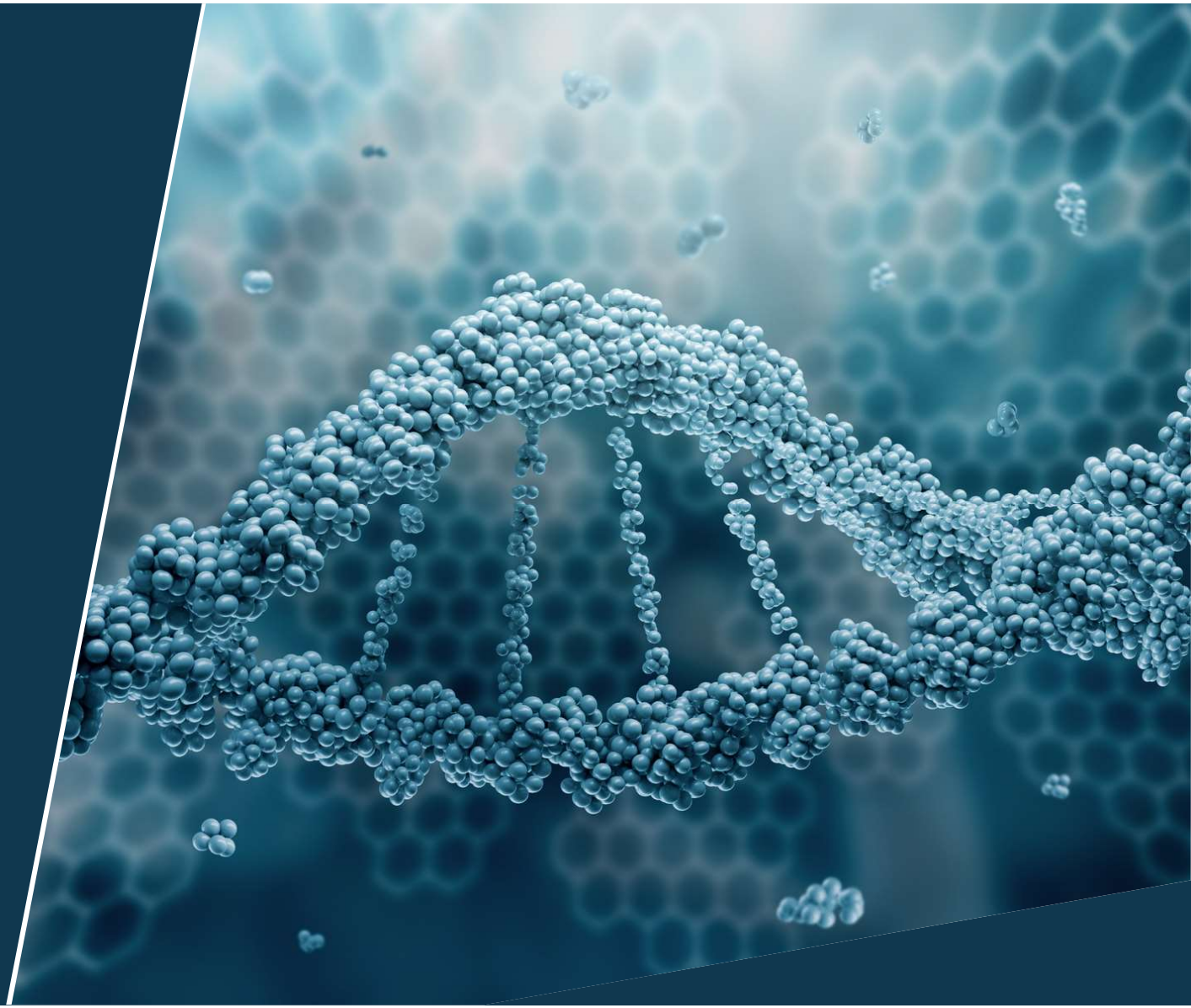
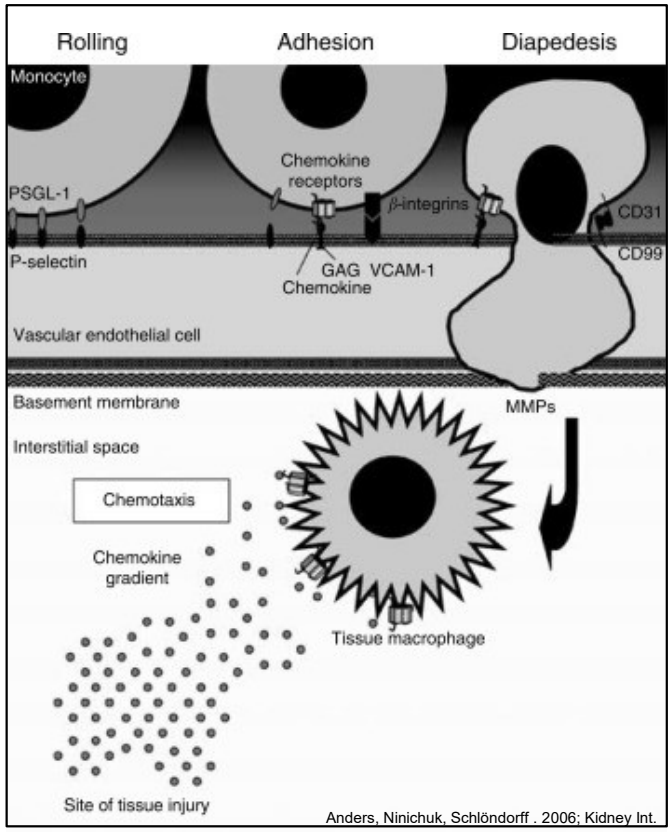


CCR1 Probe BAY-3153

Scientific rationale: CCR1- Family & Function



Anders, Ninichuk, Schlöndorff . 2006; Kidney Int.

- receptor for C-C type chemokines
- member of the rhodopsin-like subfamily of G-protein-coupled receptors
- highly expressed by monocytes/macrophages, T-cells and dendritic cells

CCR1 ligands:

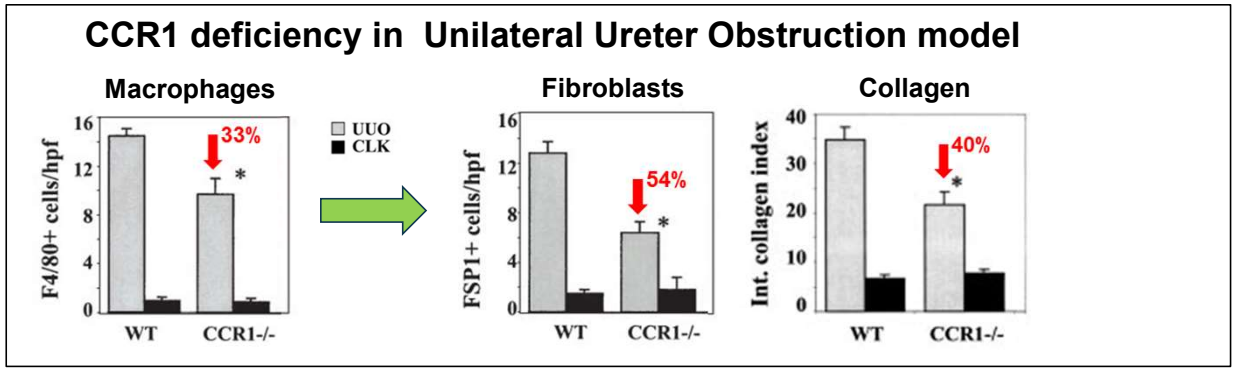
- CCL3 (MIP-1α)
- CCL5 (RANTES)
- CCL7 / CCL8 / CCL13
- CCL14/ CCL15 / CCL16
- CCL23

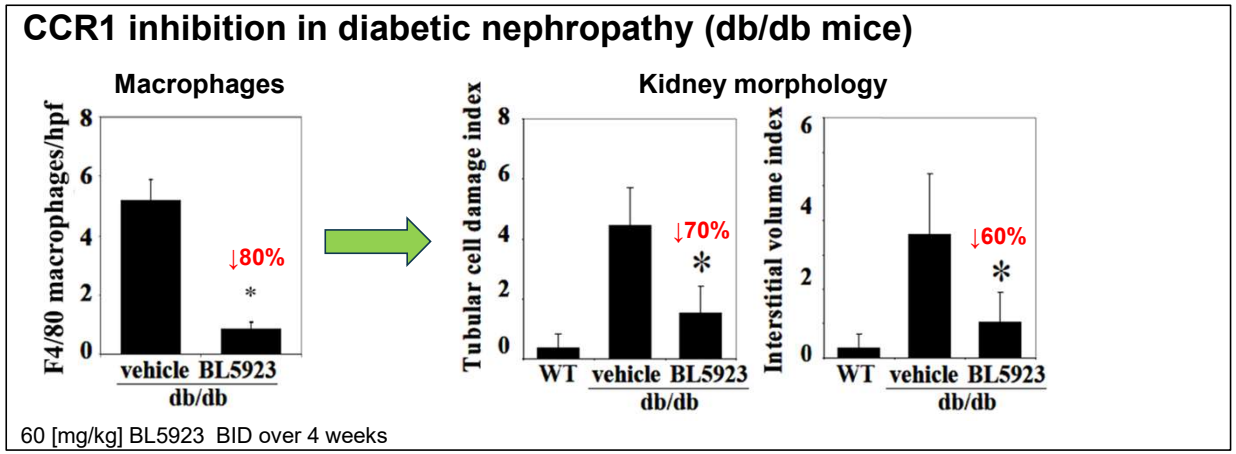
CCR1 function:

- essential for adhesion and transendothelial diapedesis of leukocytes
- leukocyte differentiation & proliferation
- T-cell activation & Th-1/Th-2 polarization

CCR1 Probe BAY-3153

Scientific rationale: CCR1 inhibition in kidney diseases





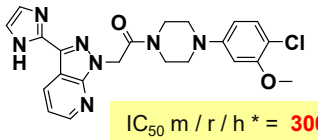
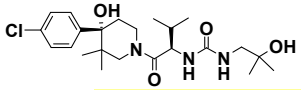
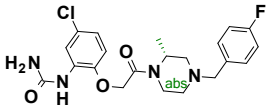
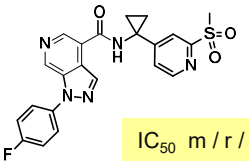
60 [mg/kg] BL5923 BID over 4 weeks

CCR1 deletion or pharmacological inhibition reduces macrophage numbers leading to reduced fibrosis and improving kidney morphology

Eis et al. 2004; J Am Soc Nephrol /Ninichuk et al. 2007; Am J Pathol

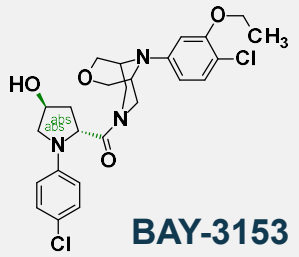
CCR1 Probe BAY-3153

Competitor compounds show only weak or almost no activity on rodent CCR1

Company & Cpd.	Structure	Indications	Devel. Status
ChemoCentryx CCX354 **(CAS 1010073-75-2)**	 IC_{50} m / r / h * = **3000 / 900 / 4 nM**	rheumatoid arthritis; Inflammatory disease. Myeloma	Phase IIa in RA, Myeloma, bone metastasis (2015-10) Safety and efficacy met. Relation to GSK ended in 2013 No development reported (2018)
Bristol-Myers Squibb BMS-817399 **(CAS 1010073-75-2)**	 IC_{50} m / r / h * = **>30 µM / >30 µM / 5.1 nM**	rheumatoid arthritis; autoimmune & inflammatory disease.	Discontinued (@2014?) Phase IIa in RA (200 + 400mg) Safety met, efficacy not met.
Berlex/BSP BX-471 **(CAS 217645-70-0)**	 K_i m / r / h ***= **215 / 121 / 1.5 nM**	multiple sclerosis; atopic dermatitis; psoriasis; endometriosis	Discontinued (2005) Phase IIa in MS. Safety met, efficacy not met.
AstraZeneca AZD-4818	structure not published; compound not available	COPD	Discontinued (2009) Phase IIa in COPD: 150 µg BID (inhalation) Safety met, efficacy not met.
Boehringer Ingelheim BI 639667	 IC_{50} m / r / h ** = low potency / low potency / **24 nM**	unknown	Discontinued, due to BMS asset was discontinued (sufficient target coverage was demonstrated).

This probe:

*** abs - absolute**



BAY-3153

IC_{50} m / r / h = **81 / 11 / 3 nM** (Ca^{2+} flux)

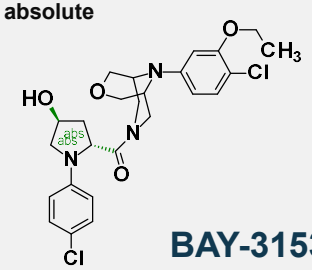
Competitor compounds show only weak or almost no activity on rodent CCR1

* in house (Ca^{2+} flux)
** literature (Ca^{2+} flux)
*** literature (^{125}I-MIP-1a displacement assay)

CCR1 Probe BAY-3153

Technical *in vitro* profile

* abs - absolute



BAY-3153

POTENCY (IC_{50} [nM])	
CCR1 (Ca2+ flux) IC_{50} rat / mice / hum.	11 / 81 / 3
CCR 3 (human)	>30 µM
CCR 2/4/5/6/7/8/9/10 (human)	>30 µM
CXCR1/2/3/4/5 (human)	>30 µM

Properties & Physchem	
LogD @ pH 7.5	3.1
Sw @ pH 6.5 [mg/L]	0.23
Vehicle for PD experiments	H_2O (50%)/ Solutol (40%)/ EtOH (10%)
MW / TPSA [g*mol / Å^2]	506 / 65
Stability (r/h plasma, 4h) [%]	ongoing

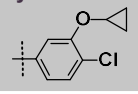
Solubility Vehicle screen:
ongoing

Solubility vehicle for PK:
H_2O (50%)
PEG400 (40%)
EtOH (10%)

in vitro DMPK Properties

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	223		537		2.4	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		-		-	
	rat hepatocytes		3.0		28	
	human hepatocytes		0.7		47	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	17	5.6	>20	7.4	0.6
CYP induction	3A4: NOEL 3333 µg/L					

Selectivity

Eurofins @ 10 µM (Panlabs results) from very close analog 	MAO-B: 73% inh. Motilin: 69% inh. Adenosin A3: 60% inh.

SAFETY

Cytotox	no data
hERG IC_{50} [µM]	9.1

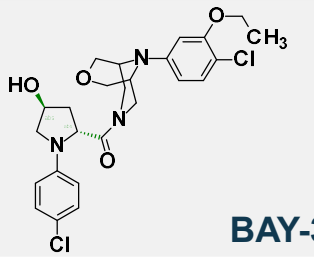
***PK, rat, iv.* bolus:**
Cl_{Plas} = 2.4
Vss = 4
$t_{0.5}$ = 2 h, MRT = 1.7 h

PK, rat, p.o:
MRT = 2.5 h
F = 19 % (1mpk)

- BAY-3153 has high *in vitro* potency and selectivity
- BAY-3153 has low solubility and high permeability

CCR1 Probe BAY-3153

Selectivity profile in more detail



BAY-3153

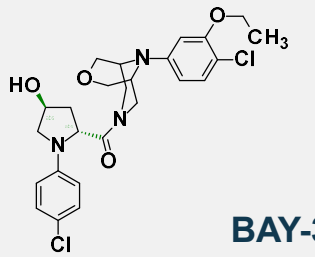
POTENCY (IC_{50} [nM])	
Biochem. IC_{50} rat / mice / hum.	11 / 81 / 3
CCR 3	>30 µM
CCR 2/4/5/6/7/8/9/10	>30 µM
CXCR1/2/3/4/5	>30 µM

- BAY-3153 was not profiled in LeadProfilingScreen Panel
- Very close analog was tested at Eurofins @ 10 µM
  - activity of this analog was reported for
    - MAO-B: 73%
    - Motilin: 69%
    - Adenosin A3: 60%
- Kinase Panel: initiated

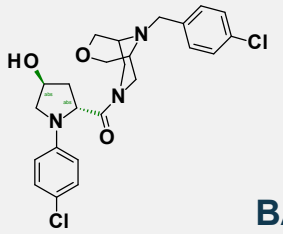
- BAY-3153 is a highly selective CCR1 inhibitor
- Close analog of BAY-3153 was profiled in LeadProfilingScreen Panel showing weak activity on three additional targets
- Eurofins hits are regarded as being irrelevant for the macrophage modulating function of the CCR1 probe

CCR1 Probe BAY-3153 and negative control

Selectivity profile in more detail – PDSP screen data



BAY-3153



BAY-173

PDSP Screen KI [nM]

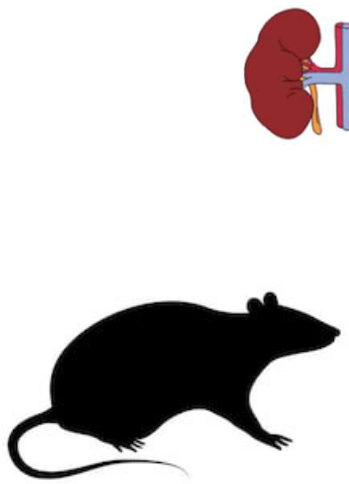
BAY-3153	
Sigma 1	1828,52
Sigma 2	1476,05
KOR	3005,38

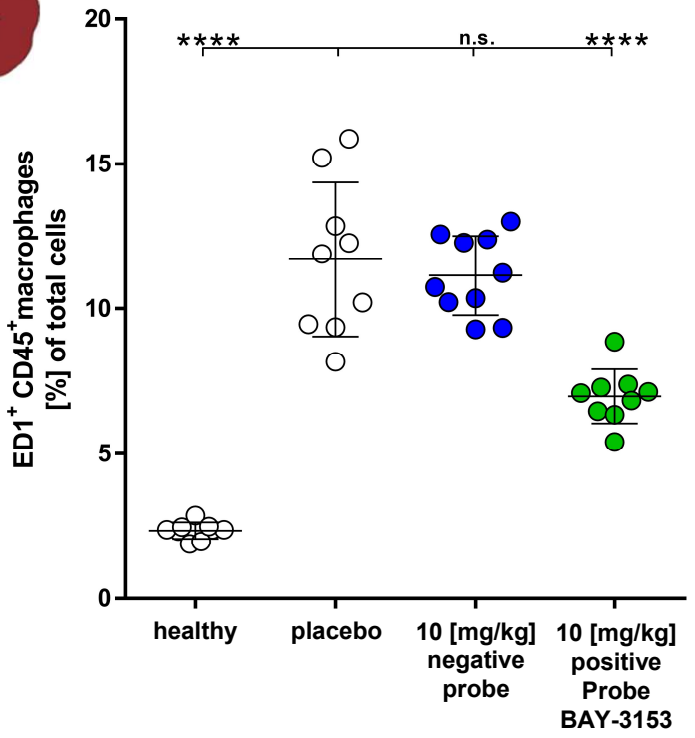
BAY-173	
Sigma 1	8245,18
Sigma 2	2169,7
Alpha2B	>10,000
SERT	1460,83

- BAY-3153 and BAY-173 were profiled in PDSP screen (thank you Brian Roth)
- Sigma1/2 and KOR were identified as off targets, KI was determined in detail

CCR1 Probe BAY-3153

Mechanistic *in vivo* assay



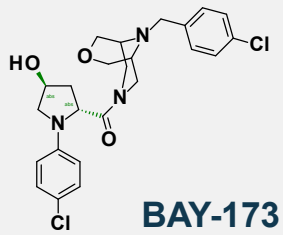


Data are mean ± SD */**/***/****= significant with p<0.05/0.01/0.001/0.0001 vs. placebo (One-way ANOVA followed by Dunnett´s Multiple Comparison post-hoc analysis)

Reduction of infiltrating macrophages after renal ischemia/reperfusion injury (I/RI) in rats

CCR1 Probe BAY-3153

In vitro profile of Negative Control BAY-173



BAY-173

POTENCY (IC_{50} [nM])	
CCR1 (Ca2+ flux) IC_{50} rat / mice / hum.	8500 / nd / 1200
CCR 3 (human)	no data
CCR 2/4/5/6/7/8/9/10 (human)	no data
CXCR1/2/3/4/5 (human)	no data

Properties & Physchem	
LogD @ pH 7.5	3.2
Sw @ pH 6.5 [mg/L]	ongoing
MW / TPSA [g*mol / Å^2]	476 / 56
Stability (r /h plasma, 4h) [%]	ongoing

in vitro DMPK Properties

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	no data		ongoing		ongoing	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		no data		no data	
	rat hepatocytes		no data		no data	
	human hepatocytes					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	no data					no data
PXR	no data					

Selectivity

Eurofins @ 10 µM (Panlabs results)	no data

SAFETY

Cytotox	no data
hERG IC_{50} [µM]	no data

- BAY-173 has low in vitro potency.

CCR1 Probe BAY-3153

Summary / Conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 100 nM (IC_{50}, Kd)	**Surpasses criteria**; high potency in biochemical CCR1 assay with IC_{50} 3 nM
Selectivity within target family: goal is > 30-fold	**Surpasses criteria**; inactive on CCR3, > 30-fold selective vs other relevant target family members
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	Close analog of BAY-3153 (O-cPr derivative, see page 5) was profiled in LeadProfilingScreen Panel showing activity on MAO-B, Motilin and Adenosin A3 receptor. Eurofins hits are regarded as being irrelevant for the macrophage modulating function of the CCR1 probe
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Surpasses criteria**;
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	**Surpasses criteria**; suitable pharmacokinetic profile for *in vivo* studies; *in vivo* efficacy on infiltrating macrophages in experimental renal ischemia/reperfusion model in mice
Neg ctrl: *in vitro* potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Surpasses criteria**; BAY-173 with 400-fold less *in vitro* potency (IC_{50} 1200 nM)

We ask for acceptance of CCR1 inhibitor BAY-3153 as chemical probe, accompanied by BAY-173 as negative control.

CCR1 Probe BAY-3153

Project Team / Acknowledgement

Medicinal Chemistry
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Toxicology
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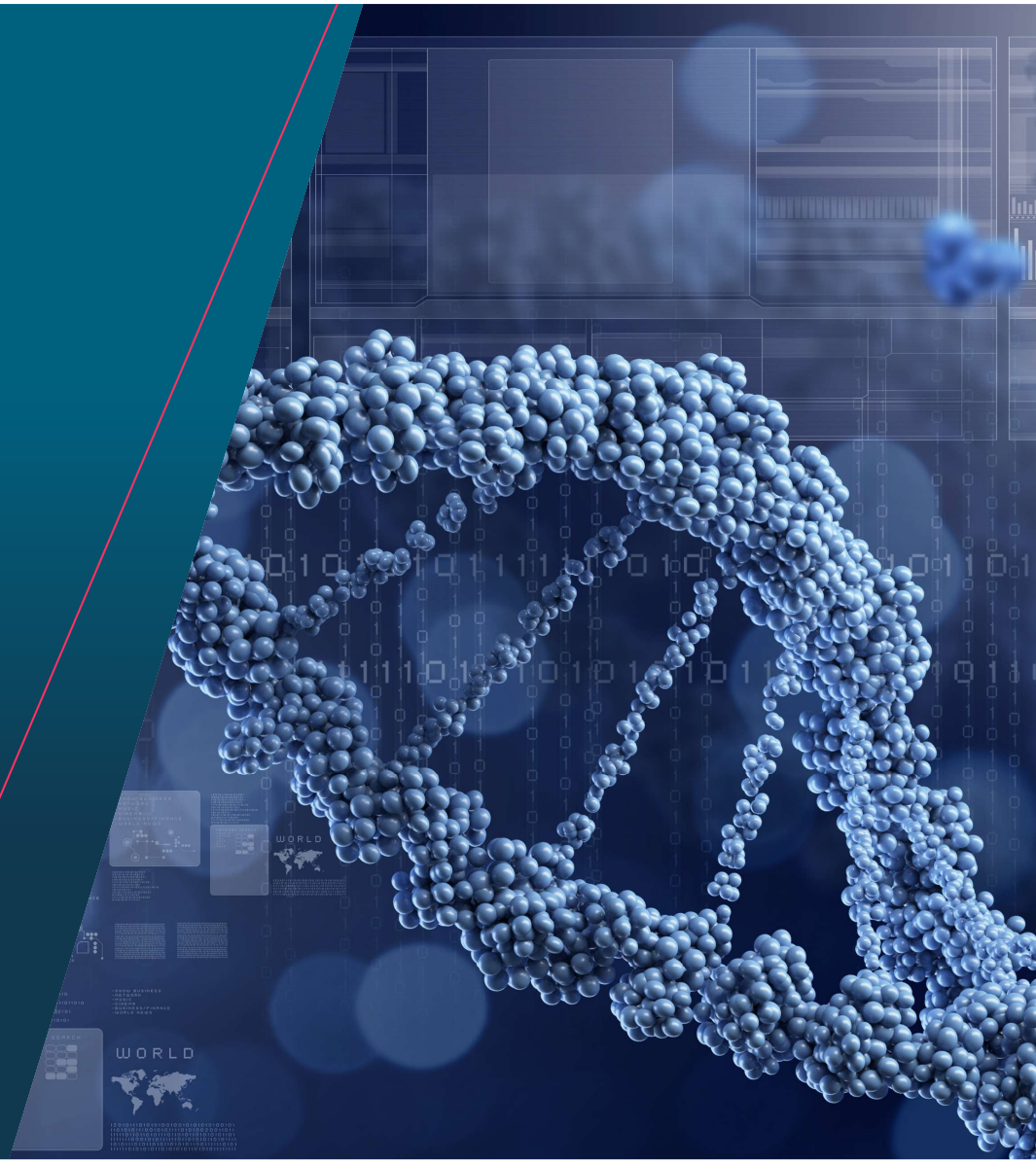
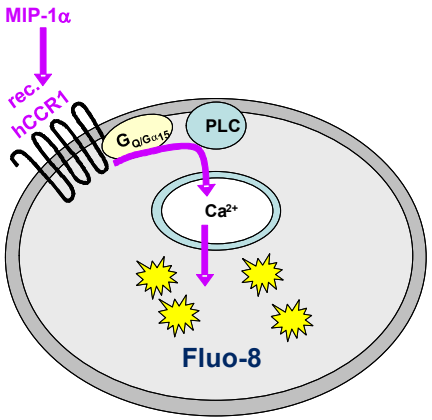
Thank You



CCR1 Probe BAY-3153

CCR1 cellular Ca^{2+}-flux assay

Schematic overview of CCR1 Ca^{2+}-flux assay:



- Coupled to Ga15, Gq
- Detection of receptor activity by Ca^{2+}-flux
- Ca^{2+} detection by Fluo-8

Description:
In vitro assay to test antagonistic activity of test compounds on human CCR1. The CCR1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant murine CCR1 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Calcium transients are detected by Fluo8 Ca-sensitive dye.

Assay Protocol:
Assay cell:
ChemiSCREEN Human Recombinant CCR1 Chemokine Receptor Calcium-Optimized Stable Cell Line, #HTS005C
Seeding:
2000 cells/well , appr. 20 hrs (37°C, 5% CO2) before start of the test, 30 µl in DMEM, 400 µM glucose with 5% FCS
Assay:
remove medium; + 25 µl Fluo-8 solution (Ca Tyrode, 10 µg/ml Brilliant Black, 2.5 µM Probenicid, 5 µM Fluo8); 30 min incubation at 37°C, 5%CO2; + 10 µl test compound solution (2 µl compound + 120 µl Ca Tyrode, 0.05% BSA); 30 min incubation at 37°C, 5%CO2; + 20µl MIP-1α solution (1 nM f.c. Ca Tyrode, 0.05% BSA); fluorescent measurement of kinetics for 180 sec.